CD80 Is Upregulated in a Mouse Model with Shear Stress-Induced Atherosclerosis and Allows for Evaluating CD80-Targeting PET Tracers

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Abstract: Purpose: A shear stress-induced atherosclerosismouse model was characterized for its expression of inflammation markers with focus on CD80. With this model, we evaluated two positron emission tomography (PET) radiotracers targeting CD80 as well as 2-deoxy-2-[18F]fluoro-D-mannose ([18F]FDM) in comparison with 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG). Procedure: A flow constrictive cuff implanted around the common carotid artery in apolipoprotein E knockout mice resulted in plaque formation. CD80 expression levels and plaque histopathology were evaluated. Serial PET/X-ray computed tomography scans were performed to follow inflammation. Results: Plaque formation with increased levels of CD80 was observed. Histologically, plaques presented macrophage-rich and large necrotic areas covered by a thin fibrous cap. Of the CD80- specific tracers, one displayed an increased uptake in plaques by PET. Both [18F]FDG and [18F]FDM accumulated in atherosclerotic plaques. Conclusion: This mousemodel presented, similar to humans, an increased expression of CD80 which renders it suitable for non-invasively targeting CD80-positive immune cells and evaluating CD80- specific radiotracers.

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Targeting plaque inflammation with PET radiotracers in a shear stress-induced atherosclerosis mouse model

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Supplementary Table

Table S1. Total cholesterol, triglycerides, HDL and LDL values (mean ± SD, after HFD onset) in ApoE KO-cuff HFD, ApoE KO-cuff ND and C57BL/6-cuff ND mice. Data are presented in Figure 1. LDL values were calculated according to [1].

	ApoE KO-cuff HFD	ApoE KO-cuff ND	C57BL/6-cuff ND
Total cholesterol [mmol/L]	39.00 ± 3.54	13.97 ± 1.52	2.50 ± 0.50
Triglycerides [mmol/L]	3.07 ± 0.61	2.29 ± 0.54	1.79 ± 0.44
HDL [mmol/L]	7.76 ± 1.82	6.71 ± 1.20	2.58 ± 0.59
Calculated LDL [mmol/L]	29.81 ± 2.53	5.98 ± 1.91	-0.89 ± 0.43

Table S2. Number of CD68-positive macrophages per section analyzed in the segments downstream (ds), upstream (us) and within the cuff and control. For each time point two different animals and two different sections at a distance of approx. 50 μm were analyzed. Few, <20 macrophages; Many, ≥20 macrophages.

	6 weeks post-surgery	9 weeks post-surgery	16 weeks post-surgery	18 weeks post-surgery
Cuff DS	Few	Many	Few - Many	Many
Cuff	None	None	None	None
Cuff US	None	Many	Few - many	Many
Control DS	Few	Many	Many	Many
Control	None	Few	None	Few
Control US	None	Many	Few	Few

Table S3. Cap thickness in μm ± STDEV of segments downstream (ds), upstream (us) and within the cuff and control. For each time point two different animals and two different sections at a distance of approx. 50 μm were analyzed. N.d., not determined.

	6 weeks post-surgery	9 weeks post-surgery	16 weeks post-surgery	18 weeks post-surgery
Cuff DS	n.d.	20 ± 8	35 ± 10	48 ± 26
Cuff	n.d.	n.d.	n.d.	n.d.
Cuff US	n.d.	13 ± 2	15 ± 1	9 ± 4
Control DS	8 ± 3	13 ± 5	11 ± 4	10 ± 4
Control	n.d.	n.d.	n.d.	9 ± 2
Control US	n.d.	26 ± 10	20 ± 11	16 ± 7

Table S4. Histopathological appearance of plaque morphology in the segments downstream (ds), upstream (us) and within the cuff and control. Appearance is based on microscopic analyzes regarding numbers of macrophage, plaque content (lipid, necrotic), cap thickness. For each time point two different animals and two different sections at a distance of approx. 50 μm were analyzed.

	6 weeks post-surgery	**9 weeks post-surgery**	**16 weeks post-surgery**	**18 weeks post-surgery**
Cuff DS	Fatty streaks	Fatty streaks	Circular vulnerable plaque	Circular vulnerable plaque
Cuff	No plaque	No plaque	No plaque, Focal fatty streaks	No plaque
Cuff US	No plaque	Circular vulnerable plaque	Circular vulnerable plaque	Circular vulnerable plaque
Control DS	Local vulnerable plaque	Local vulnerable plaque	Circular vulnerable plaque	Circular vulnerable plaque
Control	No plaque	No plaque	Fatty streaks	Fatty streaks
Control US	No plaque	Local and circular vulnerable plaque	Focal fatty streaky, circular vulnerable plaque	Circular vulnerable plaque

Supplementary Figures

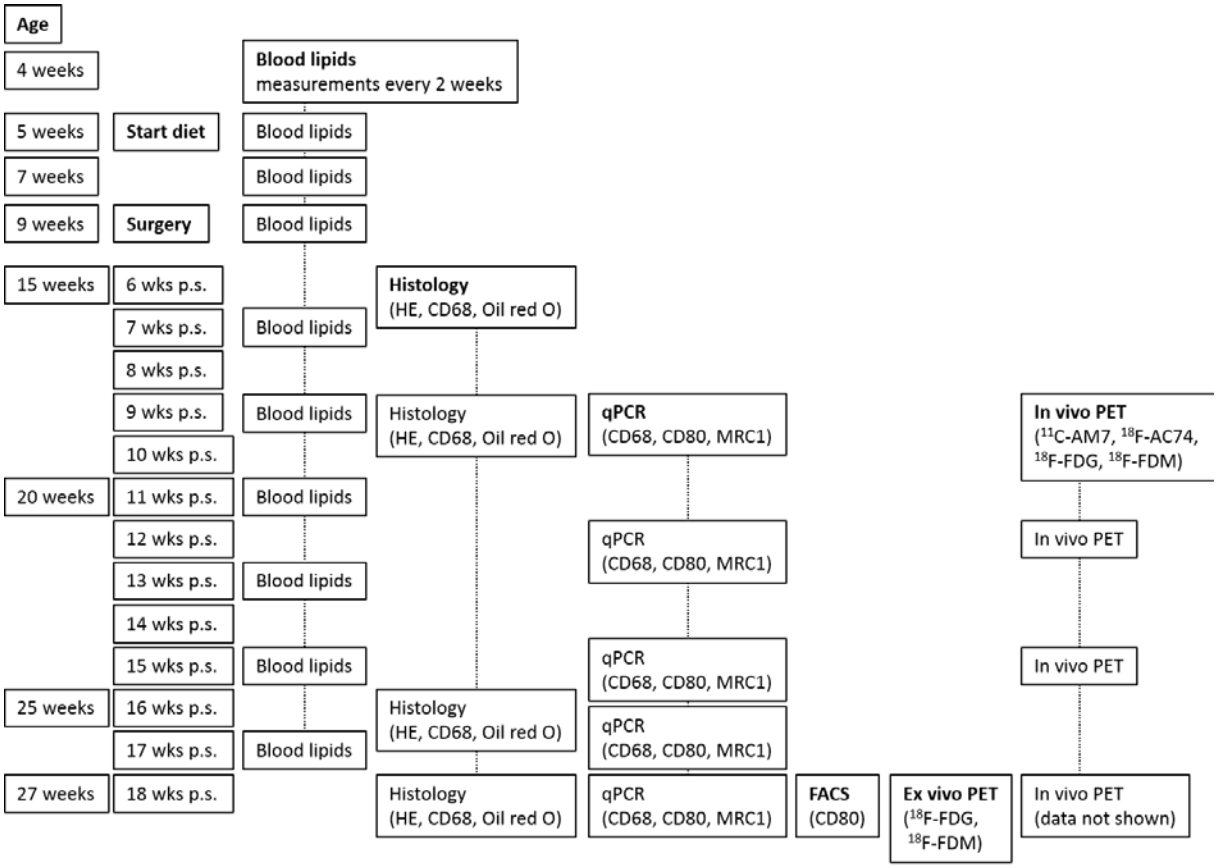

Figure S1. Workflow demonstrating type of experiments (incl. repetitions) in correlation to the age of the mice (ApoE KO and C57BL/6) and the time point post-surgery (implantation of cuff and control).

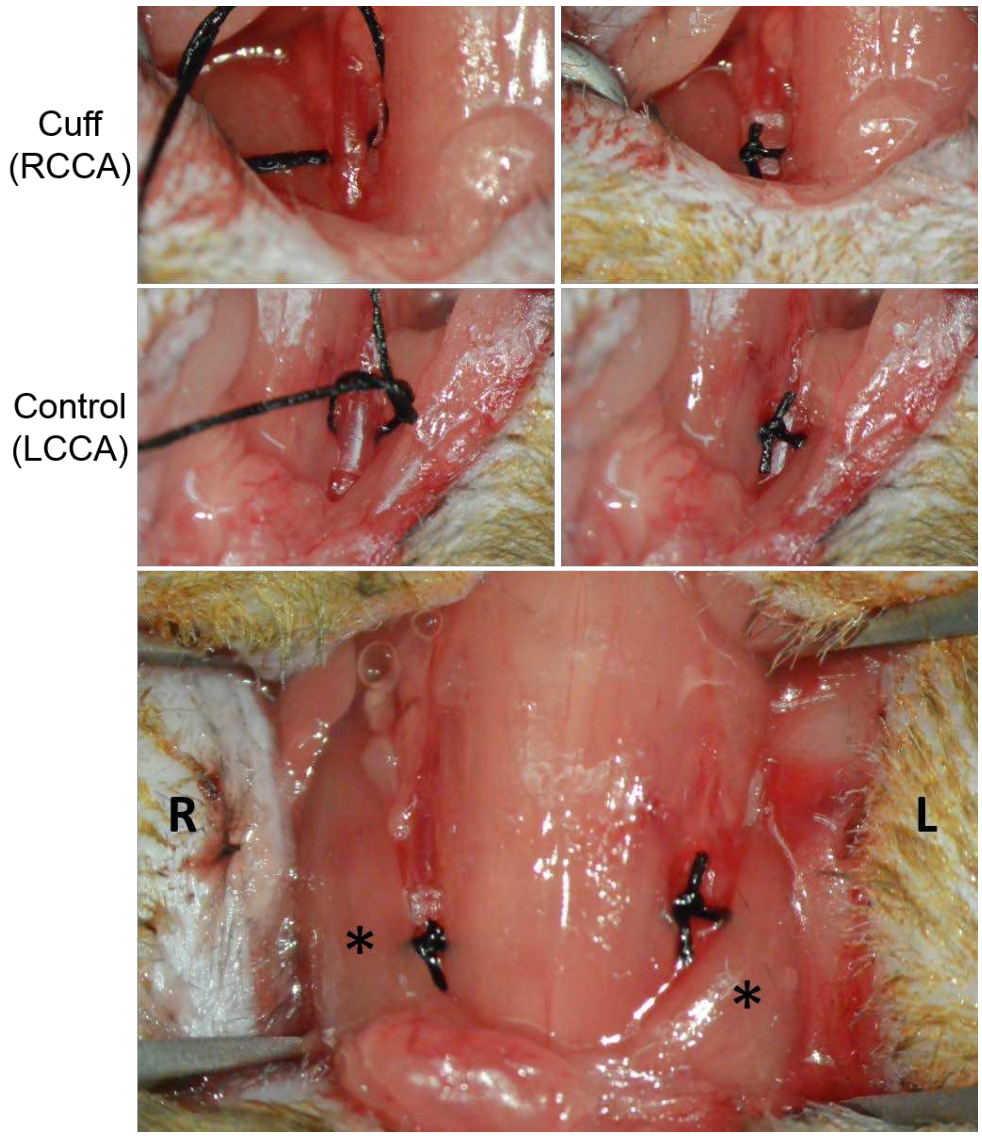

Figure S2. Photographs of a mouse undergoing implantation of a cuff around the right common carotid artery (RCCA) and a control around the left common carotid artery (LCCA). Both, cuff and control, are place right above the *musculus sternocleidomastoideus* (asterisks). R, right of the mouse; L, left of the mouse.

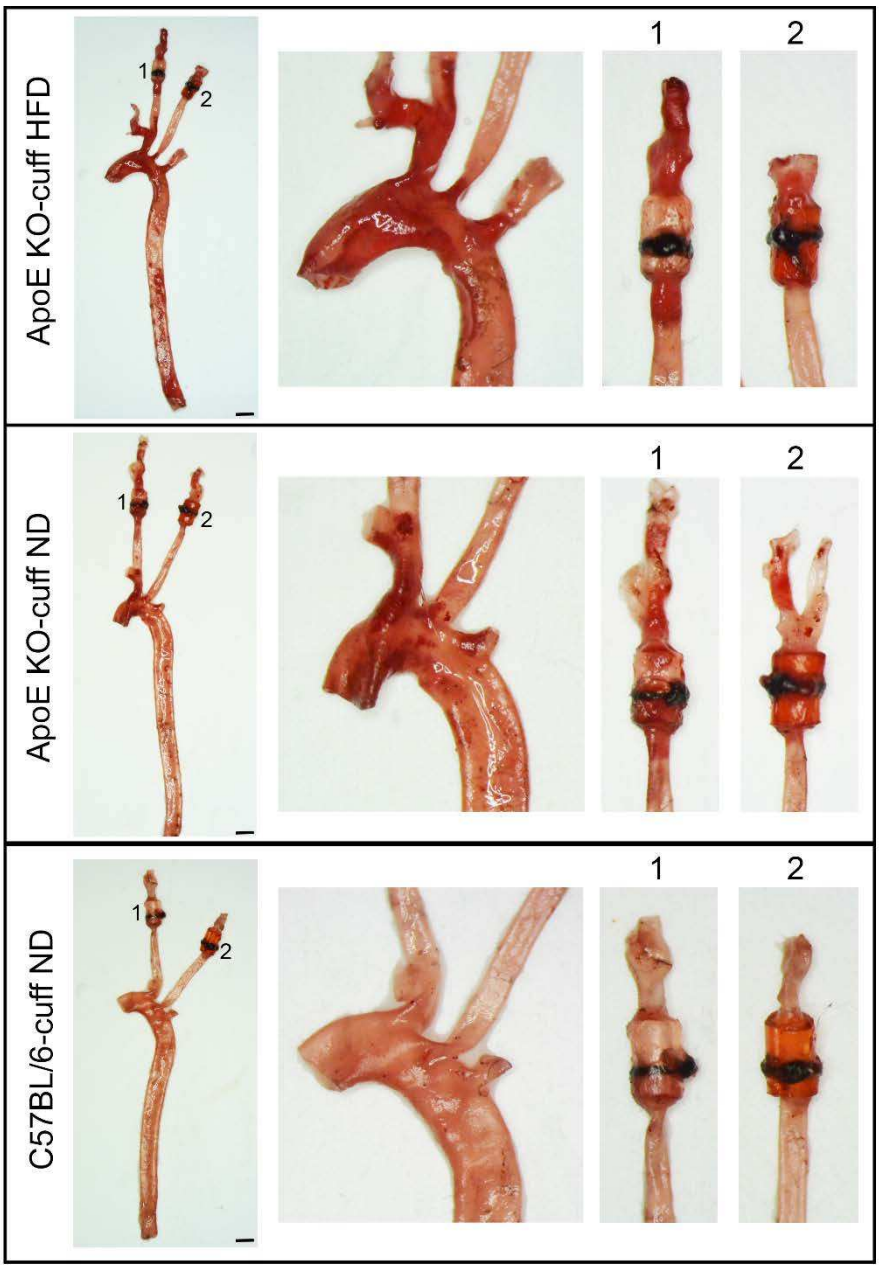

Figure S3. Representative lipid staining (oil red o) images of the aorta and carotids of ApoE KO-cuff on high fat diet (HFD), ApoE KO-cuff on normal diet (ND) and C57BL/6-cuff ND mice. Tissues were dissected 17 weeks (ApoE KO-cuff HFD, same images as Figure 2) or 18 weeks after surgery (ApoE KO-cuff ND and C57BL/6-cuff ND). Magnified images show the aortic arch, the cuff carotid and the control carotid. ApoE KO-cuff ND mouse tissue showed red staining in curvatures and branch points of the aorta, bifurcations of the carotids and a small lipid accumulation US of the cuff. No lipid staining was observed in wild-type mice. Different colors of the cuff and control are caused by differences in the material composition of the implants. Cuff and control were fixed with a black suture. 1, cuff; 2, control. Scale bars 1 mm.

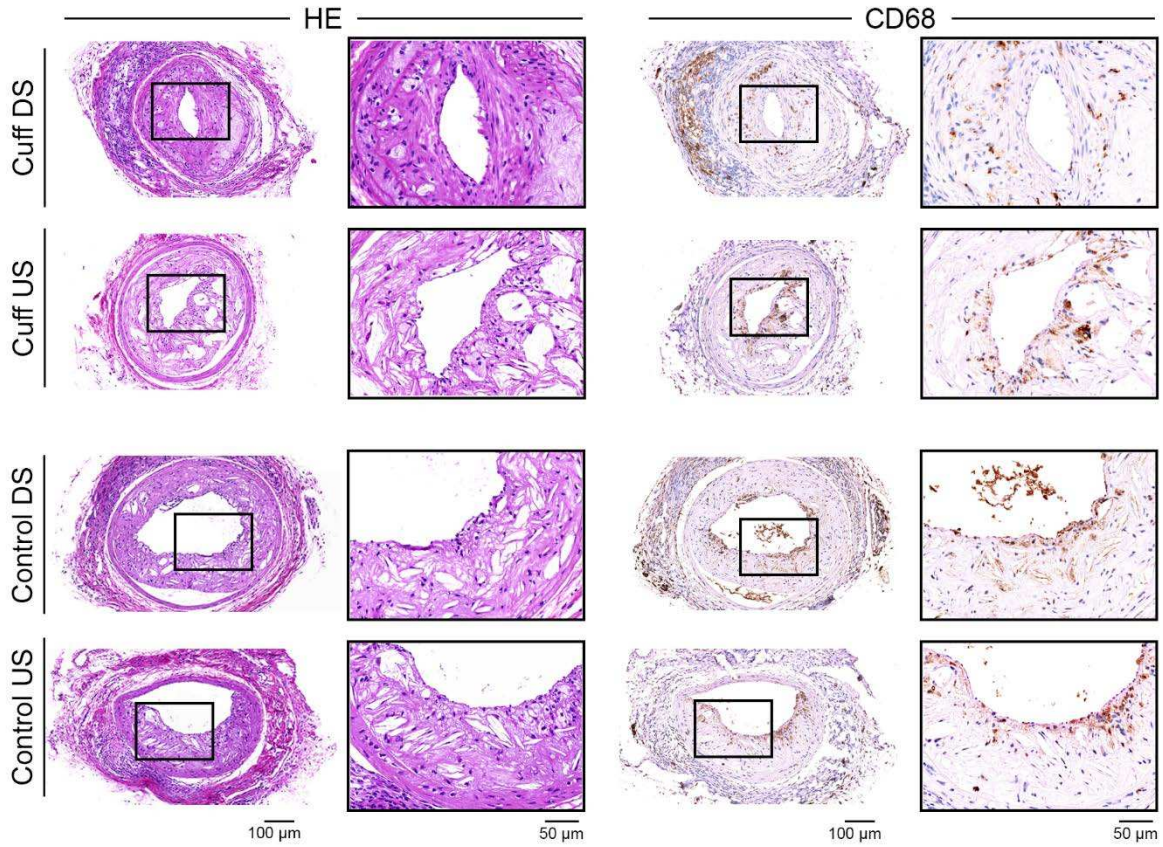

Figure S4. Representative hematoxylin/eosin (HE) and CD68 immunohistochemistry images of the segments DS and US of the cuff and the control in ApoE KO-cuff HFD mice 18 weeks post-surgery. Higher magnification images show fibrous cap thickness. The fibrous cap thickness was on average 13±5 µm, except for the plaque in the segment ds of the cuff (55±44 µm).

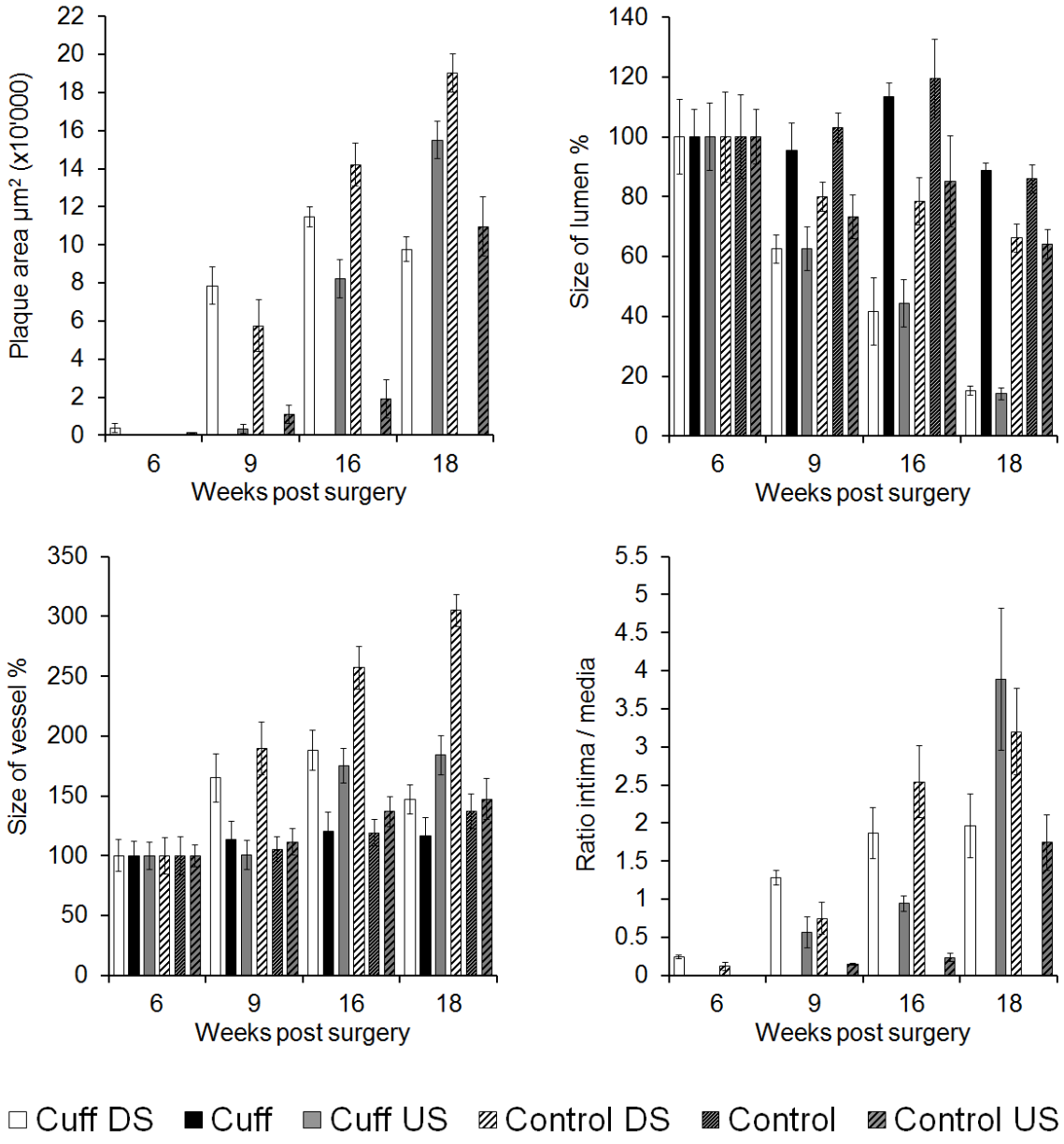

Figure S5. Quantification of plaque area (in µm²), size of lumen (100% = 52'000-56'000 µm²), size of vessel (100% = 93'000-130'000 µm²) and ratio between intima and media. For each time point two different animals and two different sections at a distance of approx. 50 µm were analyzed. Shown are the mean and standard deviations of the four samples. Absence of bars in the graph showing the ratio intima/media arise from the absence of plaques.

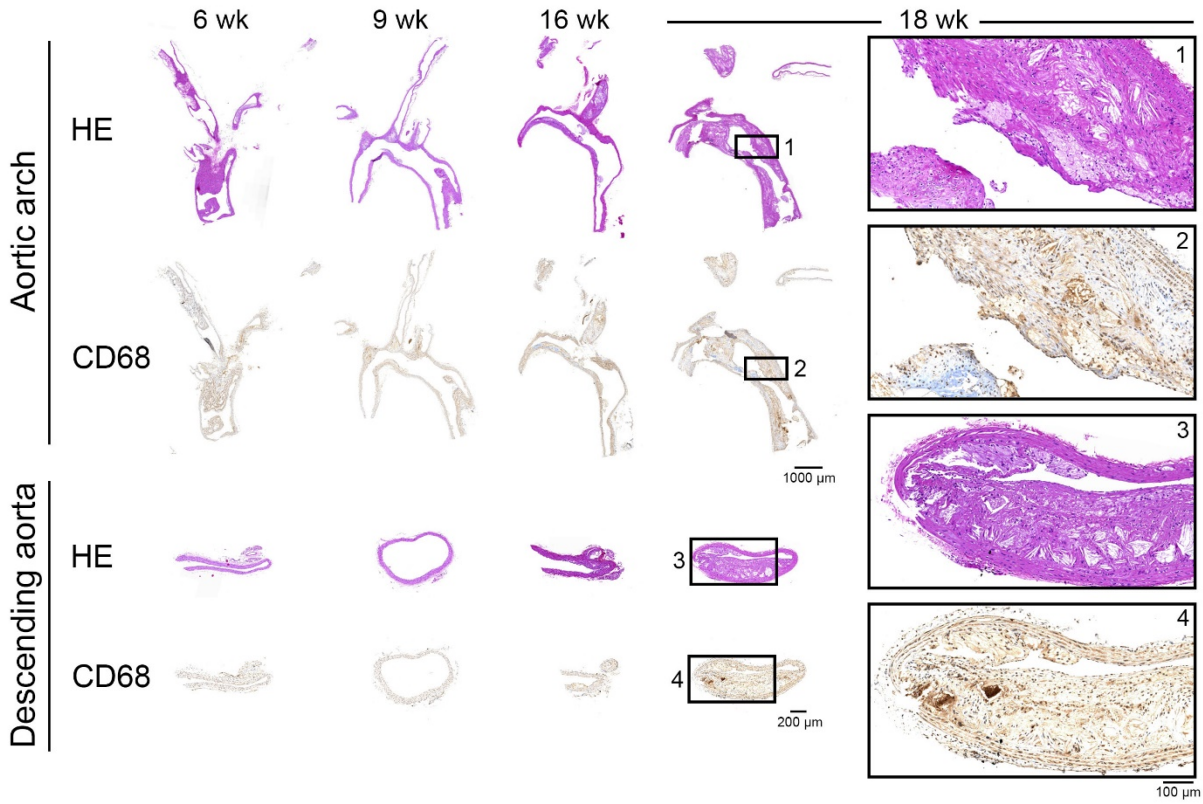

Figure S6. Representative hematoxylin/eosin (HE) and CD68 immunohistochemistry images of the aortic arch and the descending aorta of ApoE KO-cuff high fat diet mice 6, 9, 16 and 18 weeks (wk) post-surgery. Higher magnification images show large lipid-rich plaques containing cholesterol crystals and inflammatory cells within the aortic arch (1, 2) or the descending aorta (3, 4).

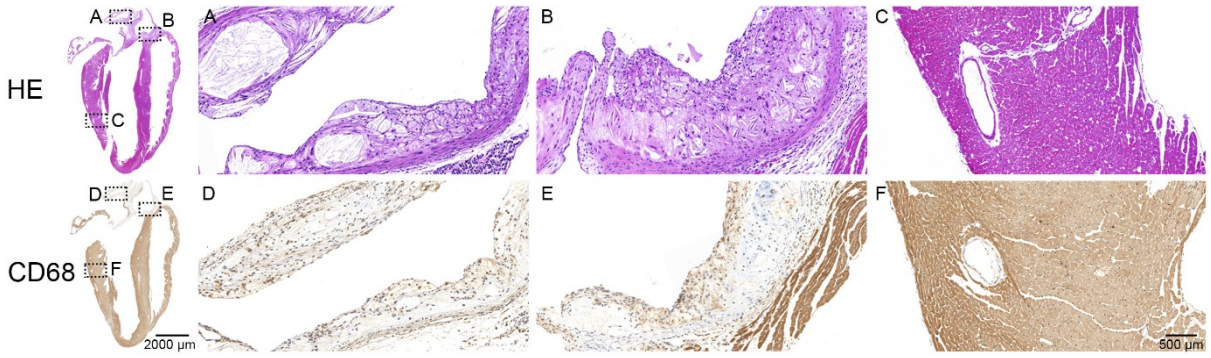

Figure S7. Representative hematoxylin/eosin (HE) and CD68 immunohistochemistry images of the heart of an ApoE KO-cuff high fat diet mouse 18 weeks post-surgery. **A+D** large arterial vessel, **B+E** aortic valve, **C+F** coronary vessel in the left ventricle. Note the intense CD68 background signal in the myocardium.

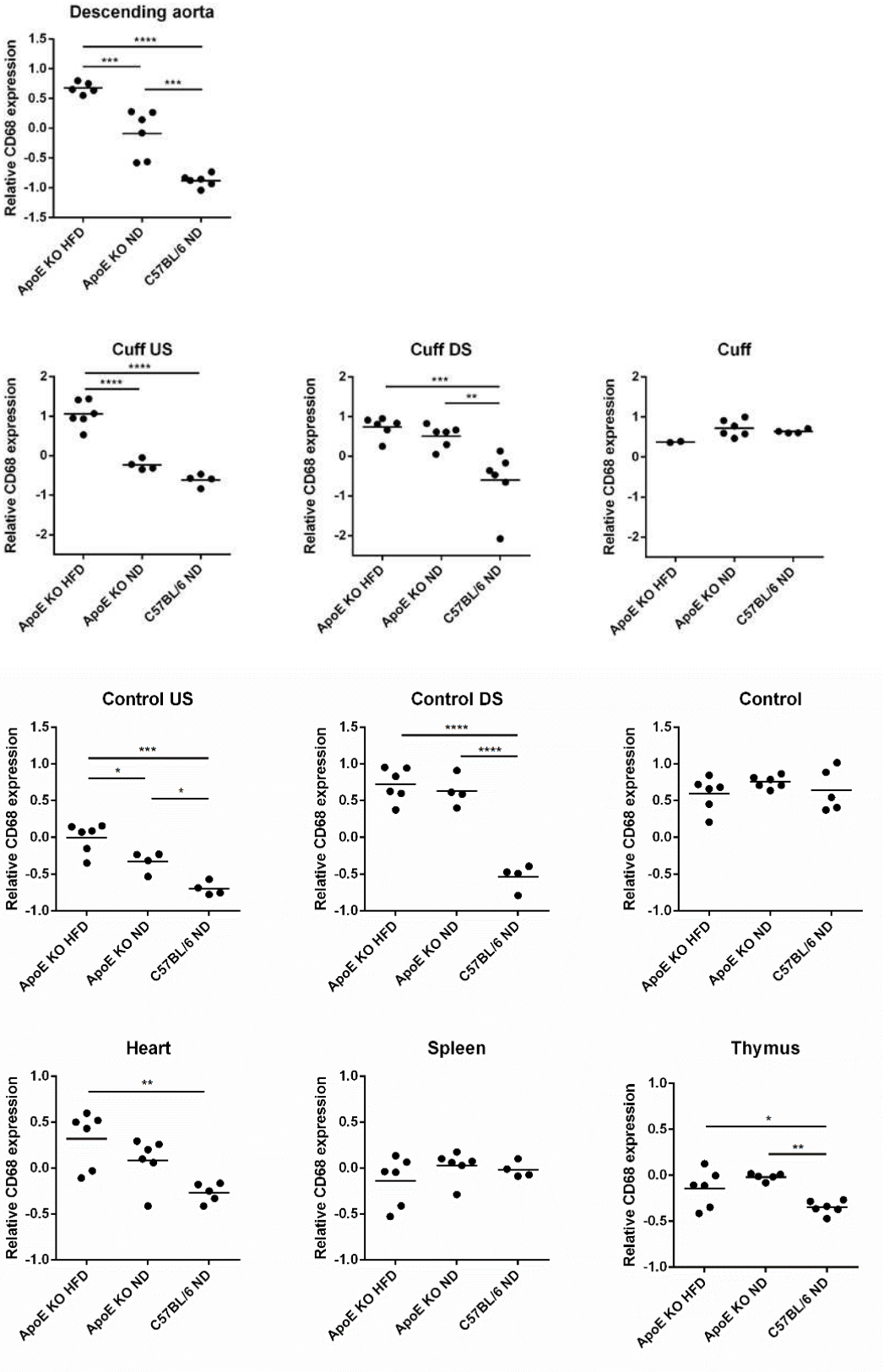

Figure S8. Relative CD68 mRNA expression in ApoE KO-cuff on high fat diet (HFD), ApoE KO-cuff on normal diet (ND) and C57BL/6 ND mice 18 weeks after surgery. Expression was analyzed in the descending aorta, cuff segments, control segments, heart, spleen and thymus. Each symbol represents the mean value of one animal per experiment (n = 2 independent experiments). Lines indicate mean values per group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

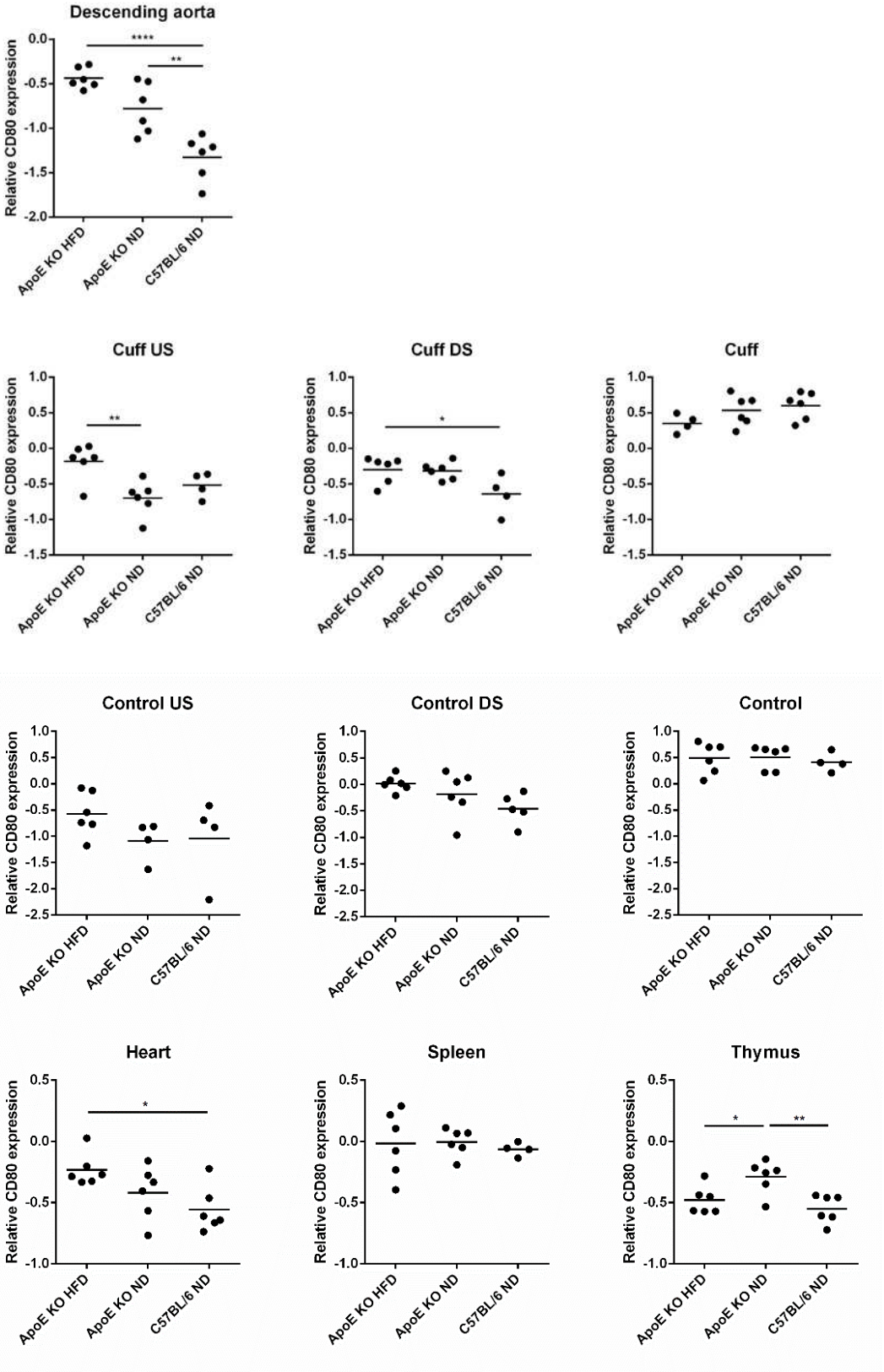

Figure S9. Relative CD80 mRNA expression in ApoE KO-cuff on high fat diet (HFD), ApoE KO-cuff on normal diet (ND) and C57BL/6 ND mice 18 weeks after surgery. Expression was analyzed in the descending aorta, cuff segments, control segments, heart, spleen and thymus. Each symbol represents the mean value of one animal per experiment (n = 2 independent experiments). Lines indicate mean values per group. * $p<0.05$, ** $p<0.01$, **** $p<0.0001$.

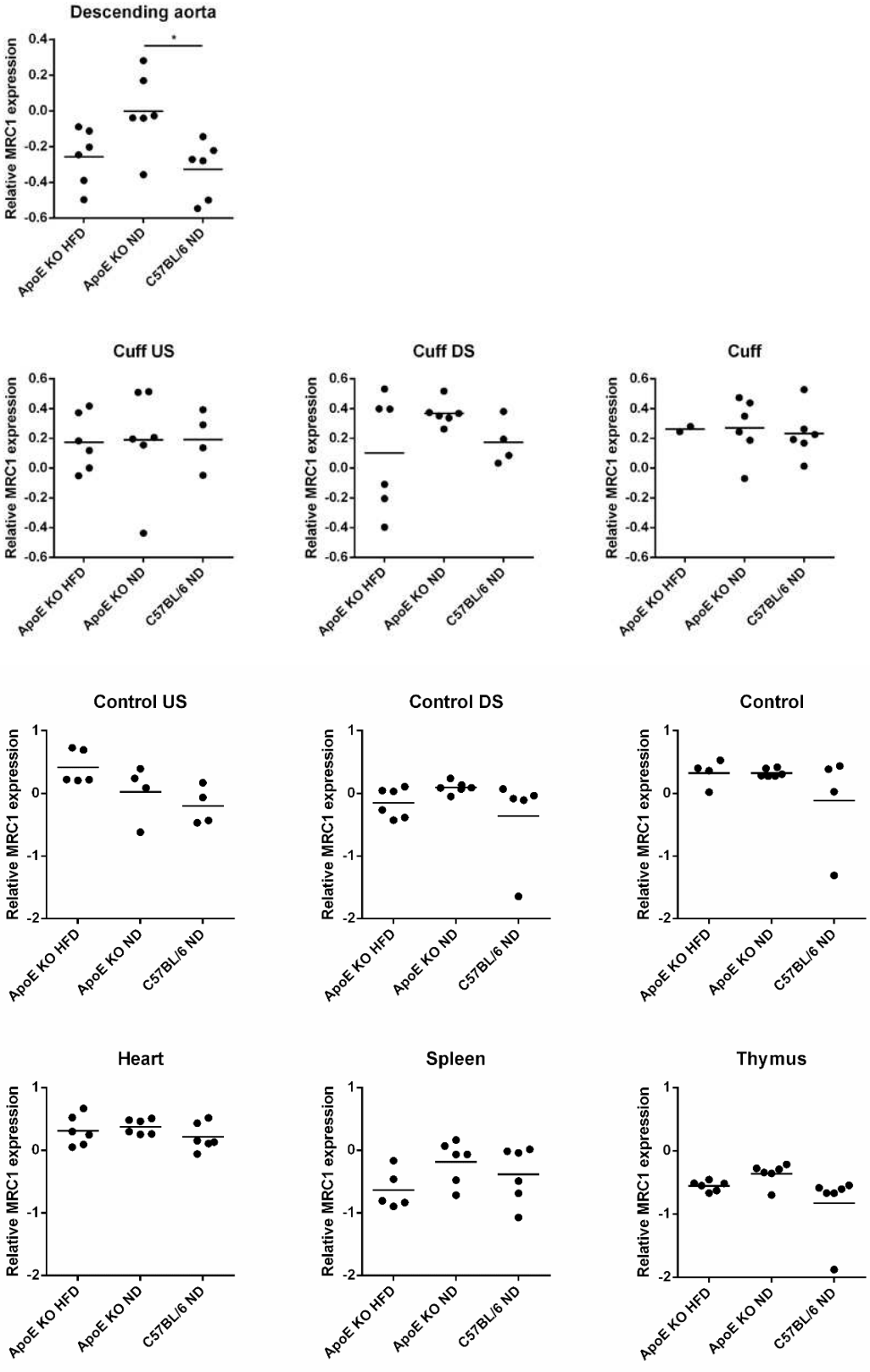

Figure S10. Relative MRC1 mRNA expression in ApoE KO-cuff on high fat diet (HFD), ApoE KO-cuff on normal diet (ND) and C57BL/6 ND mice 18 weeks after surgery. Expression was analyzed in the descending aorta, cuff segments, control segments, heart, spleen and thymus. Each symbol represents the mean value of one animal per experiment (n = 2 independent experiments). Lines indicate mean values per group. * $p<0.05$.

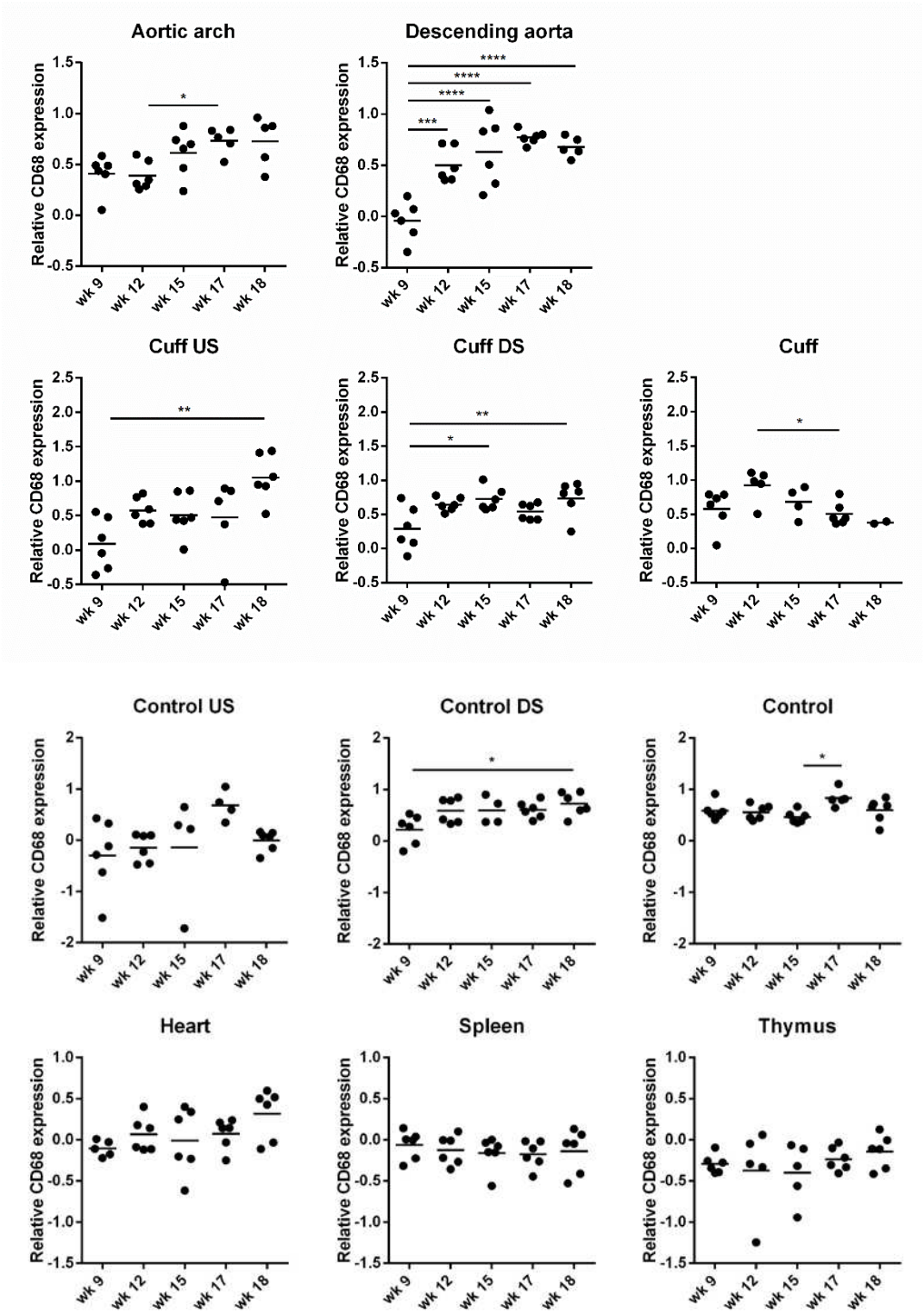

Figure S11. Relative CD68 mRNA expression in ApoE KO-cuff on high fat diet (HFD) mice 9, 12, 15, 17 and 18 weeks (wk) after surgery. Expression was analyzed in the aortic arch, descending aorta, cuff segments, control segments, heart, spleen and thymus. Each symbol represents the mean value of one animal per experiment (n = 2 independent experiments). Lines indicate mean values per group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Note that the data of ApoE KO HFD 18 weeks are in addition shown in Figure S8.

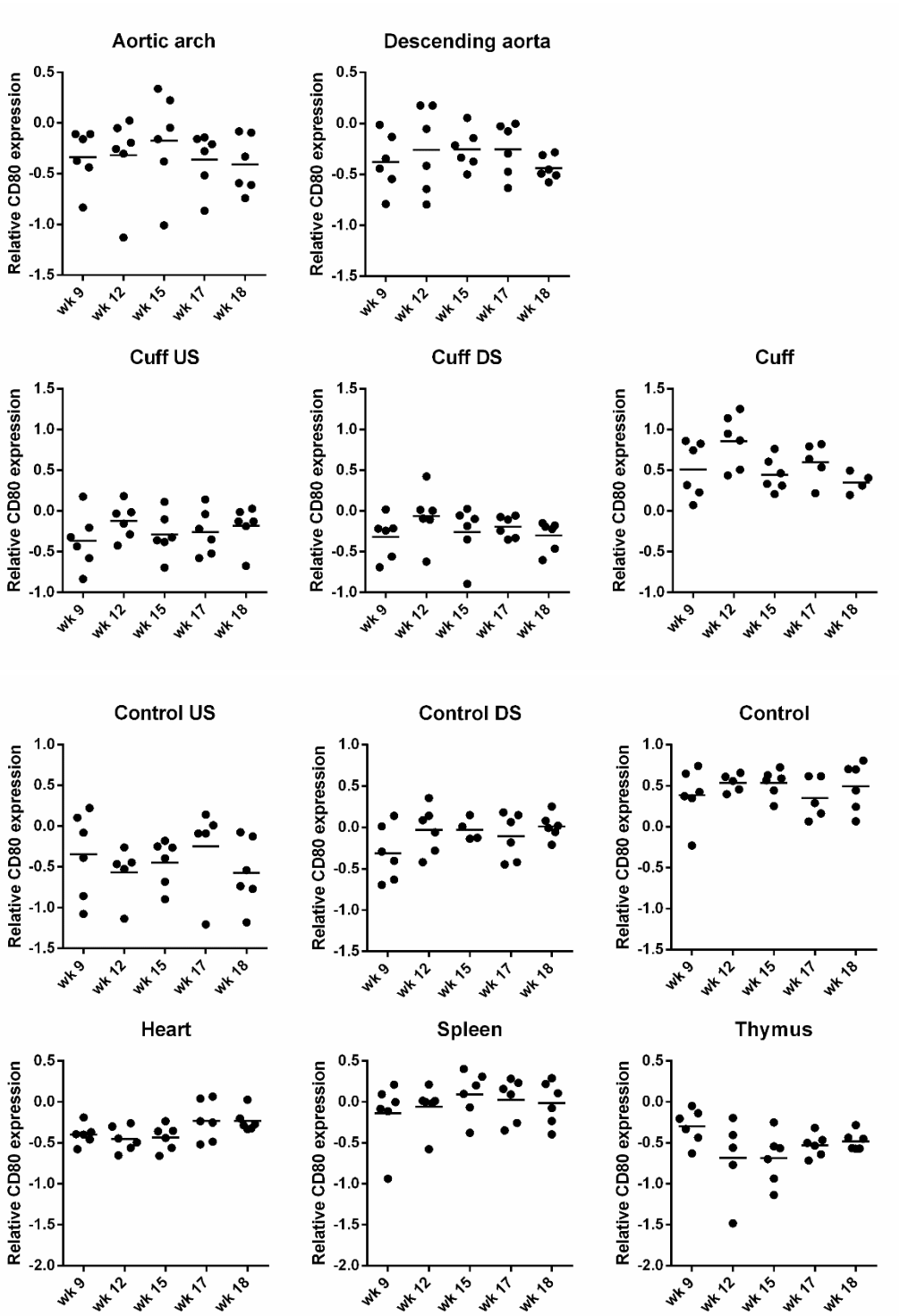

Figure S12. Relative CD80 mRNA expression in ApoE KO-cuff on high fat diet (HFD) mice 9, 12, 15, 17 and 18 weeks (wk) after surgery. Expression was analyzed in the aortic arch, descending aorta, cuff segments, control segments, heart, spleen and thymus. Each symbol represents the mean value of one animal per experiment (n = 2 independent experiments). Lines indicate mean values per group. Note that the data of ApoE KO HFD 18 weeks are in addition shown in Figure S9.

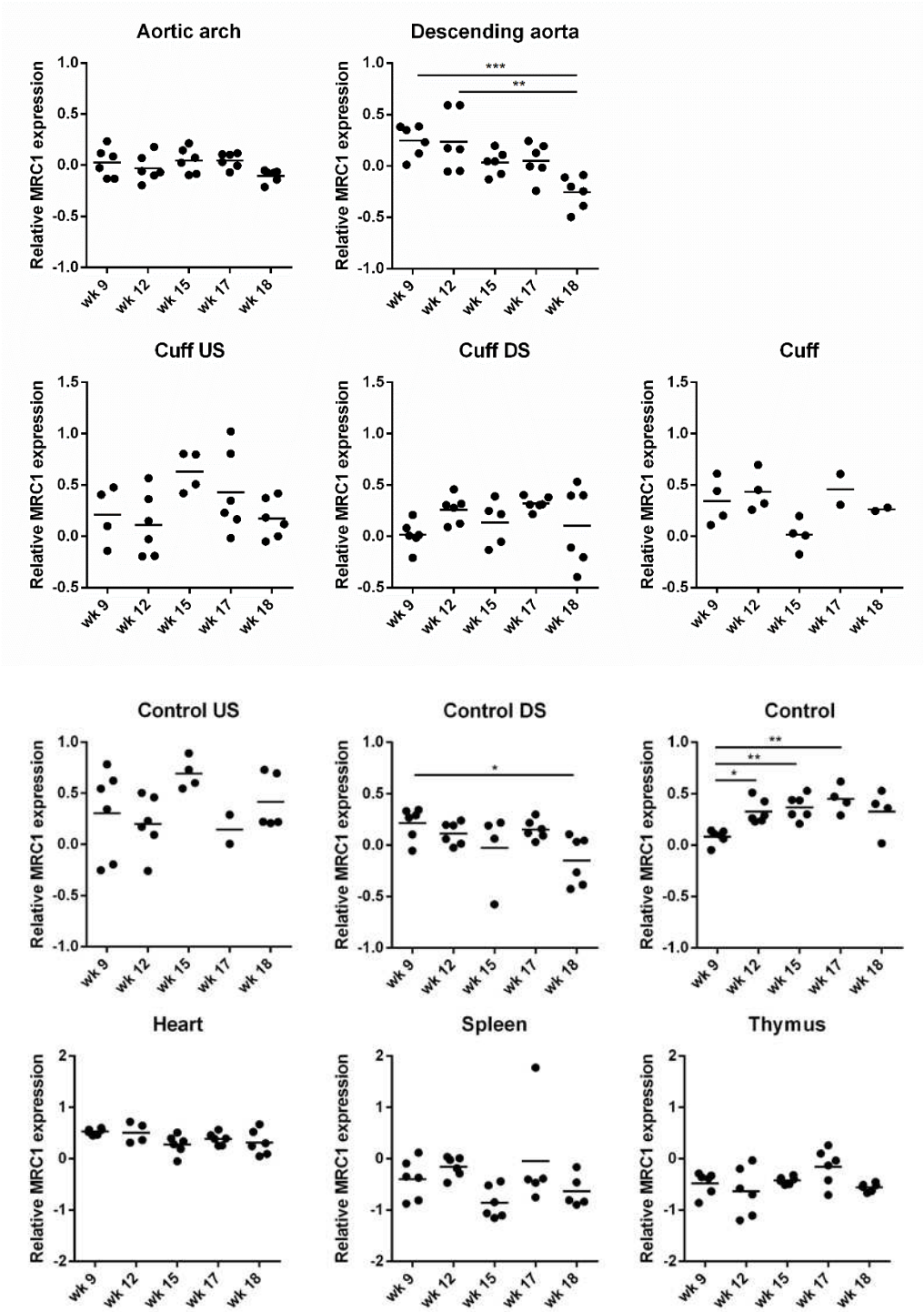

Figure S13. Relative MRC1 mRNA expression in ApoE KO-cuff on high fat diet (HFD) mice 9, 12, 15, 17 and 18 weeks (wk) after surgery. Expression was analyzed in the aortic arch, descending aorta, cuff segments, control segments, heart, spleen and thymus. Each symbol represents the mean value of one animal per experiment (n = 2 independent experiments). Lines indicate mean values per group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Note that the data of ApoE KO HFD 18 weeks are in addition shown in Figure S10.

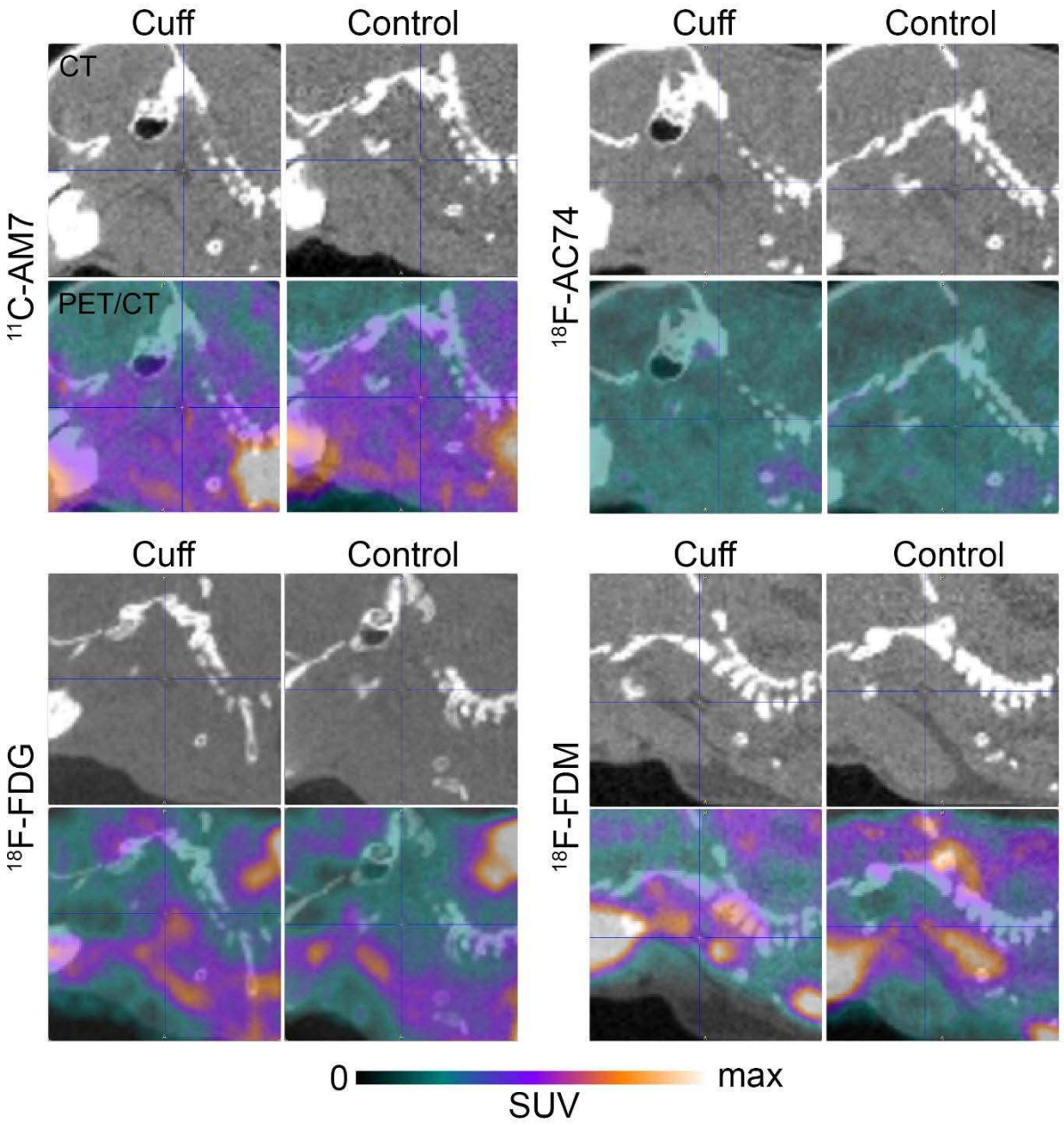

Figure S14. In vivo CT (gray scale) and PET/CT slices of the neck region of ApoE KO mice (15 weeks post-surgery) after injection of either ^{11}C-AM7, ^{18}F-AC74, ^{18}F-FDG, or ^{18}F-FDM. Crosshair indicates cuff and control as clearly visible in CT images. Maximal SUV for ^{11}C-AM7 and ^{18}F-AC74 is 0.3; for ^{18}F-FDG and ^{18}F-FDM is 0.5. Images were averaged from 1-61 min post injection of ^{11}C-AM7 and ^{18}F-AC74; and from 30-60 min post injection of ^{18}F-FDG and ^{18}F-FDM.

References

1. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.